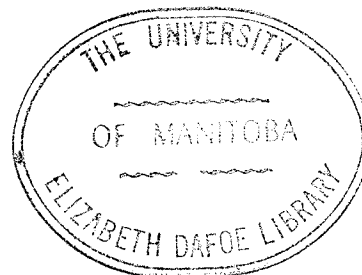SODIUM   EXCHANGES AMONG MATERNAL   AND

FETAL SODIUM COMPARTMENTS IN NORMAL

AND SODIUM-DEPLETED EWES

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Donald Charles Green

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ABSTRACT

Transfer rates of sodium were investigated in
pregnant ewes near term. The ewes were depleted of
sodium by draining the saliva from one parotid gland
for a period of seven days. A potassium supplement
was given to prevent concomitant potassium depletion.
Similar observations were made on non-sodium-depleted
pregnant ewes.

A steady-state, unconstrained, four compartment
system was proposed. The compartments were: the maternal
exchangeable sodium pool, the fetal exchangeable sodium
pool, the amniotic sodium pool, and the allantoic sodium
pool. Analysis of the data utilizing this model failed
to yield rational results. Two compartmental analysis
yielded transfer rates of sodium that were similar to
those reported by workers for other species.

Sodium depletion was observed to cause a signifi-
cant decrease of the maternal plasma and allantoic fluid
sodium concentration and a significant increase in the whole
fetal tissue dry matter and amniotic fluid volume.

The evidence indicates that the fetal system responds
to sodium depletion in a manner similar to the maternal
system.

It is suggested that the fetal urine contributes to
both the amniotic and allantoic fluids.

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INTRODUCTION

Sodium, the major cation of the extracellular fluid compartment, has been the centre of a great deal of investigation. In the normal non-pregnant animal, sodium distribution and its regulation has been rather adequately studied and reviewed by several authors. Sodium metabolism in the non-pregnant sodium-depleted animal has also been studied to a considerable extent. In relation to such conditions of pregnancy as pregnancy toxemia, oligohydramnios, and hydramnios, sodium and water metabolism in pregnancy has also been intensively studied and reviewed. However, relatively few researchers have studied the state of sodium-depletion in the pregnant animal.

In experiments on pregnant rats fed diets low in sodium it was observed that the animal severely restricted sodium losses in the urine. Although a similar response was observed in the non-pregnant controls, it was more marked in the pregnant animals. The suggestion was made that the maternal system responds to sodium depletion in such a way as to maintain normalcy of the fetal milieu.

It has been demonstrated with pregnant rats that when the mother was shown to have a sodium deficit, the fetus was able to maintain normal sodium plasma levels. Further evidence was presented to suggest that the fetus, during conditions of sodium deficiency, are able to independently regulate their urinary sodium losses in an effort to maintain homeostasis.

In studies on sodium depletion in both pregnant ewes and rats it was observed that in non-depleted animals a sodium concentration gradient existed from the mother to the fetus, while in sodium-depleted animals this gradient was reversed. These findings were interpreted as indicating that some mechanism likely exists which affords the fetal plasma sodium level some degree of independence from the maternal system.

The present experiment was undertaken to study the sodium transfer rates among the maternal and fetal sodium compartments in both normal and sodium-depleted ewes using the radioisotope sodium-22. It was thought that a study of this type may also give some insight as to the origin of the fetal fluids and possibly reflect on the mechanisms maintaining the sodium concentration gradients among the various compartments.

Sodium state of the Body

The animal body contains approximately 0.2% of sodium. About one-quarter of this is localized in the skeleton, the remainder being found in the extracellular fluids where it can undergo very active metabolism. Sodium is important in maintaining acid-base balances for it is the major cation of the bicarbonate and phosphate buffering systems of the body. Sodium makes up 90% of all the extracellular cations thus is largely responsible for the total osmotic pressure of this fluid compartment. It is also important in the maintenance of membrane potentials, being actively pumped out of cells from the intracellular fluids causing a concentration gradient for sodium and potassium to develop across the cell membrane. These gradients cause membrane potentials that are necessary for the propagation of nerve impulses, muscle contraction, and other body functions (30, 42).

The bone tissue of the animal body is the only tissue in which the sodium concentration is greater than the extra-cellular fluid (46). This bone sodium pool can act as a store for sodium and under certain pathological conditions can be mobilized and drawn upon to replenish the sodium pool of the extracellular fluid.

There is a regular dietary need for sodium due to limited storage facilities and the rapid excretion of all excesses. However, the animal body has the ability to conserve sodium when the supply is inadequate (30). Aldosterone, a steroid secreted by the adrenal cortex has been cited as being

the most important sodium retaining hormone (30, 38, 49).

Regulation of Body Sodium

While most organs of the body are at least partial-
ly concerned with maintenance of constantancy in the extra-
cellular fluid, the kidneys play a more significant role than
any other organ for regulation of the constituants. The extra-
cellular fluid characteristics that the kidneys help to maintain
are: specific concentration of different electrolytes, the
osmolarity, the total fluid volume, and the hydrogen ion concen-
tration (30). Sodium is the single most important ion that
needs to be regulated. It is actively reabsorbed in all segments
of the renal tubule and by regulating the active reabsorption
mechanism, sodium may be completely reabsorbed with essentially
none passing into the urine. On the other hand, large portions
of the sodium load of the renal tubule may fail to be reabsorbed
and be excreted in the urine.

Pitts (49) has summarized the proposed mechanisms for the
regulation of sodium: 1) hemodynamic alterations in sodium load
presented to the renal tubules, 2) changes in distribution of
filtrate among nephrons of varying reabsorptive capacities, and
3) control of completeness of tubular reabsorption of sodium by
varying the secretion rate of aldosterone. Of these mechanisms,
the last has received a great deal of support and a wealth of
evidence has accumulated indicating that a potent adrenal salt-
retaining steroid, aldosterone, is involved in the fine control
of sodium balance.

The most important action of aldosterone appears to be on the renal tubular cells (38, 49). By direct influence on these cells aldosterone promotes both retention of sodium and chloride, and the elimination of potassium ions. It should be recognized that adrenal hormones affect the renal reabsorption of only a small, yet significant fraction of the filtered sodium; the bulk of the sodium reabsorbed being independent of hormonal control (10). This was demonstrated in adrenalectomized dogs, maintained 4 days without steroid supplementation on a high sodium, low potassium diet. During the control period the percent of filtered sodium reabsorbed was 98%, thus only 2% of the filtered sodium failed to be reabsorbed. When the dogs were given adrenal cortical extracts the percent of filtered sodium reabsorbed rose to 99.9%; sodium excretion falling essentially to zero.

The rate of aldosterone secretion may vary widely in humans (38). The normal rate of secretion may in fact overlap into secretion rates which are associated with pathological conditions. Aldosterone helps protect against sodium depletion or potassium accumulation in the body. Through its sodium-retaining action aldosterone is involved in the maintenance of blood pressure and blood volume (38, 62). It would appear, that, depending on the point of view, sodium regulation could be regarded either as a primary or secondary homeostatic device in the control of the extracellular fluid characteristics. If water moves passively along osmotic gradients, and for a biological

system it is more efficient to actively move solute particles
than actively moving water, then it could be argued that the
regulation of sodium per se is in itself only a mechanism
whereby the extracellular fluid volume and consequently blood
volume and blood pressure be exactingly varied. As pointed
out by Pitts (49), increased extracellular volume is compensated
for by the excretion of water, but unless accompanied by the
excretion of sodium, the loss of water is limited by the increasing
osmolarity of the body fluids.

Hemorrhage, severe sodium restriction, and constriction
of the inferior vena cava, stimulate receptors that Pitts terms
"volume receptors". These in turn initiate salt conservation
by increased secretion of aldosterone, thereby increasing tubular
reabsorption and retention of sodium, thus restoring the extra-
cellular fluid volume. Contrasted to this is expanded extra-
cellular fluid volume reducing aldosterone secretion, thus reducing
sodium reabsorption leading to reduced extracellular fluid volume.
It is quite likely that a number of receptor organs participate
in volume regulation and it is also quite likely that the central
nervous system, which the majority of investigators agree to be
a part of the diencephalon region, plays an integrative role
(4, 38).

The role of the nervous system itself in the regulation
of electrolyte and water excretion remains unsettled. Bradley
(7) points out that denervation of the kidney leads to an increa-
sed excretion of sodium and other osmotically active solutes.

Also increased urine flow is evident with renal denervation suggesting that the renal nerves have a direct influence on renal tubular reabsorption. However, renal denervation undoubtedly affects the renal hemodynamics. Whether the increased sodium excretion evident upon renal denervation is due to some mechanism at the tubular level or due to hemodynamic changes would be difficult to ascertain from the evidence presented by the author (7).

Control of Aldosterone. Aldosterone is secreted by the zona glomerulosa of the adrenal cortex. This tissue retains its functional integrity even in hypophysectomized animals, thus demonstrating it is not obligatorily dependent on the presence of the pituitary hormone, adrenocorticotrophic hormone (ACTH), although ACTH is known to stimulate aldosterone secretion (19, 38, 49). Hypophysectomized animals will increase secretion of aldosterone in response to volume depletion as do normal animals (49).

Control of aldosterone through the integrative center probably involves several mechanisms (49). However, the one "mechanism gathering the greatest amount of evidence"is outlined as follows: Impulses to the sympathetic centers of vascular control are relayed to the kidney and cause vasoconstriction of the afferent arterioles. Renin is liberated from the juxtaglomerular apparatus which acts on the angiotensinogen in the blood. This leads to the formation of angiotensin I, which under the influence of an enzyme system is

hydrolyzed in the peripheral circulation to angiotensin II, which then stimulates the zona glomerulosa to release aldosterone (38, 49).

Sodium Depletion in the Non-Pregnant Animal

Generally sodium depletion, produced either by saliva drainage or restricted sodium intake, gives rise to a decrease in the total osmotic pressure of the extracellular fluid. To compensate the kidneys excrete water resulting in a decrease in the extracellular fluid volume. Plasma concentrations of sodium and chloride decrease; also plasma volume is decreased leading to hemoconcentration and increased specific gravity and hematocrit values of the blood. The reduced blood volume leads to a fall in the blood pressure and in severe cases to circulatory failure (14).

Denton (14, 15), and Goding and Denton (27) observed in sheep the clinical symptoms of sodium depletion by draining the parotid saliva. By supplementing the animals diet with 600 mEq $NaHCO_3$ per day they were able to maintain the animal in good condition indefinitely. They observed a striking response of the parotid saliva composition to sodium depletion. When the sodium supplement was withdrawn there was a decrease in the sodium to potassium concentration ratio of the saliva from a normal value of 18 (180 mEq Na/l. to 10 mEq K/l.) to a value as low as 0.06 (10 mEq Na/l. to 180 mEq K/l.) as well as a decrease in the saliva flow rate. The Na:K ratio and

saliva flow rate could be returned to normal by replacing the sodium supplement in the diet. It was found that variations in the blood supply to the gland could account for a portion of this decrease in the Na:K salivary ratio, but increased saliva flow rates in non-depleted sheep had no effect on the Na:K ratio. Increasing the saliva flow rate in sodium depleted sheep by infusion of acetylcholine tended to offset the effects of sodium depletion by both increasing saliva flow and saliva Na:K ratio. Their observations on the non-depleted animal appear contradictory to reports by other workers. Cotes and Wright (10) observed in sheep , and Burgen (9) observed in humans, a definite relationship between the saliva flow rate and the ionic composition in the normal animal. They observed that increased saliva flow rate was associated with an increase in the concentration of sodium and bicarbonate and a corresponding decrease in potassium and phosphate. While Denton's experiments did not reveal the specific mechanism, he concluded that a local glandular factor was involved in the parotid response to sodium depletion. However, this "local factor" only accounted for about 25%-50% of the decrease in the salivary Na:K ratio. Denton (16) later observed that sodium depletion in the absence of adequate potassium intake had a severe impact on the condition of the sheep. The saliva flow rate decreased very quickly and the animal went off its feed within two to four days. Parotid saliva Na:K ratio fell even though the plasma potassium concentration decreased. The mean

arterial plasma potassium concentration in normal animals
as reported by Denton (16) was 4.4 ± 0.4 mEq/l. During
sodium depletion with adequate potassium intake plasma
potassium concentration rose to 5.6 ± 0.8 mEq/l. Denton
(16) also observed the effects of water depletion on the fall
of parotid salivary Na:K ratio during sodium depletion. Water
intake was restricted and supplementary potassium was given
(200 mEq K/day) to prevent concurrent potassium depletion. It
was found that the plasma sodium concentration increased due to
reduced plasma volume, while at the same time the saliva Na:K
ratio fell at the same rate as when water depletion was not
involved. Resumption of ad libitum water caused a large fall
in the plasma sodium concentration with little or no influence
on the parotid Na:K ratio. Water depletion plus sodium depleti-
on resulted in a more marked reduction in saliva flow rate and
in total less sodium depletion due to a more rapid decrease of
saliva flow than in animals that were depleted of sodium with
unrestricted water intake. The author suggests that such a
finding does not exclude the possibility that the mechanism
determining the parotid response reacts to changes in the sodium
concentration of the plasma. If this were the case, then according
to the findings of Munro et al (46) one could expect depletion
rates between younger and older animals to be different. They
observed that the bone sodium in young rats was more easily
mobilized and more severe depletion was required to cause plasma
sodium changes.

Denton concluded that an electrolyte-active adrenal hormone was largely responsible for the reaction of the parotid saliva to sodium depletion. Goding and Denton (26, 27), administered 5-10 mg. of desoxycorticosterone (DOCA) and 25 mg. of cortisone daily to bilaterally adrenalectomized sheep with unilateral parotid fistula receiving supplementary sodium. This therapy maintained the animals in good condition with the volume and electrolyte pattern of the parotid saliva remaining normal. When the adrenal hormone was withheld the plasma Na:K ratio fell and large sodium losses occurred via the urine. However, the saliva Na:K ratio remained unchanged regardless of the negative sodium balance and the marked decrease in saliva flow rate. This was associated with the characteristic signs of adrenal insufficiency in sheep (27). With replacement of the adrenal hormones the parotid saliva Na:K ratio fell to a level consistent with the degree of sodium depletion. If during sodium depletion the adrenalectomized sheep were given gradually increasing doses of DOCA up to six times basal, the parotid response was similar to that of normal sheep during Na depletion. This demonstrates a requirement for the adrenal hormone that is necessary for the parotid gland to undergo the normal response to sodium depletion. Results of these experiments led the authors to conclude that there are only two major factors responsible for the parotid response to sodium depletion in the normal sheep. These are an increased secretion of an electrolyte-active adrenal steroid, presumably

aldosterone, and a local parotid factor associated with decreased secretion rate.

Denton and Sabine, as reported by Denton (17) observed the behavior of sodium deficient sheep and found them to exhibit a wide variety of behavorial changes. These involved the skeletal musculature with stamping and bleating, the excitation of medullary centers as evidenced by profuse salivation, increased blood pressure, cardiac rate and respirations. The extent of behavioral change was related to the degree of sodium deficit and following sodium injections these manifestations were greatly reduced or disappeared.

Sodium Depletion in the Pregnant Animal

In the past few years experiments have been conducted to observe the effects of sodium deficiency in the pregnant animal comparing them with the pattern of sodium depletion that is known to occur in the non-pregnant animal.

Kirksey and Pike (35) investigated the effects of variations in salt intake on normal pregnant rats. The rats were fed three levels of NaCl; low salt, control, and high salt diets, with all diets containing equal potassium levels. Similar diets were fed to non-pregnant rats. Pregnant animals fed the low sodium diet showed a general languor and debility, ate less food and gained less weight than those on the control or high sodium intake. Sodium and potassium retention was greater in pregnant groups than in the non-pregnant groups. Marked conservation of urinary sodium was

observed for all animals on the low sodium diets. In
a similar study Kirksey, Pike and Callahan (36) observed
maternal plasma sodium concentration to decrease slight-
ly as a result of pregnancy while the potassium concentra-
tion was unaltered. A significant decrease in sodium and
increase in potassium concentration levels of the plasma
occurred in pregnant animals fed the low sodium diet.
Muscle sodium concentrations were slightly lower in pregnant
than in non-pregnant groups but the potassium concentrations
were unchanged. The pregnant group on low sodium demonstra-
ted a 34% decrease in muscle sodium and a 4% increase in
potassium concentration plus a significant increase in
muscle moisture content. Bone sodium decreased in the
pregnant group on low sodium but potassium and moisture
content of bone were not affected by dietary sodium or
pregnancy. Fetal plasma sodium, and potassium levels,
were not affected by the sodium intake of the mother,
despite a marked decrease in the sodium and increase in the
potassium concentration of the maternal plasma in pregnant
animals on the low sodium diet. That fetuses of this group
of animals appeared able to maintain normal sodium plasma
levels suggested to the authors the operation of a mechanism
by which the sodium ion can move against a concentration
gradient. Amniotic sodium and potassium levels were not
statistically related to maternal sodium intake suggesting
to the authors a reduction in fetal urinary sodium excretion

may be a mechanism by which the fetus of the animal on low sodium intake is able to maintain normal plasma sodium levels. From the observation that non-pregnant rats fed the same low sodium diet did not show any observable effects on sodium and potassium concentrations of tissues and fluids, the authors concluded that the marked electrolyte changes in the pregnant rats were an attempt by the maternal system to maintain homeostasis of the conceptus.

Wardlaw and Pike (66) investigated the granulation of the juxtaglomerular cells and the fluctuation of the zona glomerulosa layer of the adrenal cortex in pregnant rats fed low, control, and high sodium diets with similar diets fed to non-pregnant rats. They observed that both pregnancy and low sodium diets led to increased granulation of the juxtaglomerular cells and increased zona glomerulosa width, with the pregnant sodium-deficient group showing a greater response to the stimulus than the pregnant control group. If these observations are indicative of an active aldosterone reaction, then it is clear that both normal pregnancy and sodium depletion in pregnancy elicit an aldosterone type mechanism in the maternal system.

Stewart and Welt (61) produced potassium deficits in pregnant rats by feeding diets deficient in potassium and by peritoneal dialysis. It was found that fetal tissue was not adversely affected by potassium depletion of the maternal tissue although an absolute reduction in the fetal potassium

pool was evident. The authors suggest that an active
protective mechanism could be inherent in the placenta in-
volving some form of active transport; or alternatively a
simple chemical gradient for potassium may exist between
the maternal and fetal plasma as a result of rapid uptake
from the extracellular fluid by the rapidly growing fetal
tissue.

Phillips and Sundaram (48) observed the effects of
sodium depletion in pregnant ewes at different stages of
gestation. The maternal changes they observed were consist-
ent with previous observations of sodium depletion in non-
pregnant sheep. There was a decrease in the saliva Na:K
concentration ratio as well as a significant decrease in
maternal plasma sodium and potassium levels. The maternal
mean sodium plasma concentration decreased by a value of
14.8 mEq/l. and potassium by value of 0.4 mEq/l. which also
demonstrated a concomitant potassium depletion. They also
observed a 35% reduction in plasma volume with a 16% decrease
in body weight. A significant difference was observed between
the fetal plasma sodium levels of the depleted and control
groups which were 143.0 and 137 mEq/l. respectively, but there
was no significant difference between the plasma potassium
levels of the two groups. Amniotic fluid sodium concentrations
were significantly different between the control and depleted
groups (124.5 and 110.0 mEq/l. respectively). No significant
difference was observed for amniotic potassium. Sodium

depletion had no discernible effect on sodium or potassium
of the allantoic fluid. It was observed by the authors that
in the control animals a sodium concentration gradient existed
from mother to fetus but in the depleted ewes this gradient
was reversed. The authors interpreted this to indicate that
some mechanism likely exists which affords the fetal plasma
sodium level some degree of independence from the maternal
plasma sodium level.

Watanabe, Meeker, Gray, Sims and Solomon (67) determined
aldosterone secretion rates in normal human pregnancy by tracer
analysis and found the aldosterone secretion rate to be elevated
during the last trimester of gestation while the metabolism of
aldosterone was unaltered. They found the secretion of aldosterone
to be influenced by the level of dietary sodium. Apparently the
aldosterone secretion rate was inversely related to the amount of
sodium consumed. This elevated aldosterone level could be due
to the demands of an increased blood volume observed during
pregnancy (29).

Streeten, Rapoport, and Conn (60) demonstrated the existence
of a slowly exchangeable sodium pool in normal human subjects and
in patients with primary aldosteronism. It was observed that the
group with the elevated aldosterone secretion rates had a more
slowly expanding sodium pool which was interpreted as being indicative
of a reduced bone sodium exchangeability, due to an increased
aldosterone titre; that is, aldosterone inhibits sodium exchange
with the bone sodium pool. This was further substantiated by

Rooner, Streeten, Lewis, Stevenson, and Conn (56) who demonstrated reduced sodium and potassium uptake by compact bone of adrenalectomized rats treated with aldosterone, indicating important extrarenal effects of aldosterone in regulating the transfer of electrolytes between bone and extracellular fluids. From the work cited (56, 60, 67) it may be reasonable to draw the inference that in the maternal system of pregnant animals, due to increased aldosterone secretion, there would be decreased sodium mobilization from the bone sodium pool. Under these conditions the pregnant animal may react more severely to an acute sodium deficit than a non-pregnant animal.

Fetal Adrenal and Kidney Function

Due to the prevailing concept of the inter-relationship between the kidneys and adrenal cortex with respect to electrolyte balance in the adult animal body, it is difficult to delineate separately the function of these two organs in the fetal animal. It has been established that the fetal adrenal cortex can exert endocrine influence on fetal liver function through the secretion of glucocorticoids (18). It has also been shown that human fetal adrenals are able to synthesize several biologically active C_{21} and C_{19} steroids (18). Frandsen and Stakeman (23), in studies with humans to determine the sites of production of estrogens during pregnancy, suggested that the fetal adrenal may be a site for production of a steroid precursor of estrogen which is further metabolized by placental tissue to estrogen.

Such a mechanism would necessitate functional adrenals operating in the fetus.

Christianson and Jones, as reported by Bentley (5), failed to find evidence that fetal rats produced adrenocortical hormones that affect their electrolyte metabolism.

Jones, Jarrett, Vinson and Potter (11) detected adrenal activity in fetal lambs near term. The lambs were delivered by caesarian section, care being taken to avoid rough handling of the umbilical cord to prevent interference with fetal circulation. The fetus was kept moist and warm and heparin was administered to the fetus via the jugular vein. Cortisol and corticosterone were sought in the fetal adrenal venous blood and peripheral fetal blood by isotope dilution techniques. The results suggested that the fetal adrenal gland, like other endocrine glands, may be active in the later part of fetal life.

Glaz and Sugar (25) demonstrated decreased aldosterone production in rats due to blocking of the synthesis of aldosterone from corticosterone by heparin. If this mechanism operates in the fetal lamb then conceivably Jones et al (11), may have underestimated the capacity of the fetal adrenal.

Eguchi (20) made detailed studies on the fetal mouse adrenal cortex. In this study he pointed out that by the 15th day of fetal development the adrenal cortex is arranged in the characteristic three layers and that the critical period of the onset of secretory activity is probably between the 18th and 19th

days of fetal development. Adrenal growth continues until
the last day of pregnancy but is reduced at birth.

Schapiro and Geller (57) stated that the new-born
rat does not respond to stress with pituitary-adrenal activa-
tion which is not due to either ACTH deficiency or to a failure
of the fetal adrenal to respond, thus inferring that the adrenal
is capable of responding. The fetal adrenals do in fact exhibit
weight changes under conditions designed to influence pituitary
ACTH secretion (57).

Fetal renal function has been studied in relatively few
species, with observations in the lamb, rat and human contributing
the greater part of knowledge. Alexander, Nixon, Widdas and
Wohlzogen (3) studied the development of renal function in the
fetal lamb. The observations were made on the metanephric
kidney which is thought to be functional in the fetal lamb at
40 days, while the mesonephric kidney is completely degenerate
at 55 days of age. This study was on the metanephric kidney
from 61 to 142 days gestation age. These workers considered
fetal renal tubular function only with respect to the development
of water and electrolyte reabsorption. It was found that at 61
days fetal age only 25% of the filtered water was reabsorbed and
that the level did not exceed 66% until 130 days. Eighty to
ninety percent reabsorption of water occurred between 130 and
142 days. Sodium and chloride were quite similar; at 60 days
fetal age 60% of the filtered sodium and chloride were reabsorbed.
This increased to 80% at 131 days and further increased to 95%

at 140 days fetal age. Potassium reabsorption was 80%
at 61 days fetal age and apparently failed to increase
with fetal age. The combined effects of these different
rates of water and electrolyte reabsorption resulted in
the formation of hypotonic urine between 61 and 130 days
and subsequently hypertonic urine between 130 and 142 days.
The authors concluded that the metanephric kidney tubule is
capable of osmotic work in the sense of active electrolyte
reabsorption, while it is only late in gestation that the
fetal kidney begins to develop the adult capacity of active
water reabsorption and the production of hypertonic urine.

Boylan, Colburn and McCance (6) studied renal function
in fetal guinea pigs. They made direct comparisons of the
maternal and fetal urines and found that the osmolar concentra-
tion of the maternal urine was always higher than that of the
fetal urine. However, the latter was always hypertonic to
serum which is opposite to what has been observed in the
human fetus and the fetal lamb. Urea was always higher in
the maternal urine and this was thought largely responsible
for the difference in total osmotic concentration. Chloride
in the fetal urine was quite variable but tended to rise and
fall with the chloride concentration in the maternal urine.
The authors make no suggestion as to the significance of this
observation.

Fetal Fluids and Placental Transfer

Malan, Malan and Curson (41) conducted an extensive

study of the amount and nature of the fetal fluids in sheep
at different stages of gestation. They found that in early
pregnancy the allantoic fluid volume was greater than the
volume of the amniotic fluid, while towards term the amniotic
fluid volume rapidly increased and at term there was no obvious
difference between the two fluid volumes. The authors also
pointed out that the sodium concentration of the amniotic fluid
exceeded the potassium concentration at all stages of gestation.
In the allantoic fluid they found that the sodium concentration
decreased while potassium concentration increased to exceed the
sodium concentration with increasing fetal age.

McDougall (43) examined the composition of fetal fluids
of sheep at different stages of gestation. The allantoic fluid
was variable in composition but increased in potassium content
as gestation proceeded. The amniotic fluid was much more constant
in composition and had a higher sodium and chloride content than
the allantoic fluid. The amniotic potassium concentration was
within the range of values for this constituent in the allantoic
fluid. The changes in the allantoic fluid suggested to the authors
an increasing excretion of fetal urine as gestation proceeds,
implying that the composition of the allantoic fluid reflects the
developing metabolic activity and functional efficiency of the
fetal kidney. McDougall conceded that Jacques (1902) was correct
in concluding that the fetal urine was excreted successively via
the urachus alone, the urachus and urethra, and finally the urethra
alone, as gestation proceeded. The mean sodium and potassium

concentrations of the amniotic fluid were 124.8 and 13.1 mEq/l. respectively. By comparing the amniotic fluid composition with that of the maternal and fetal serum, the author concluded that the amniotic fluid arises as a transudate of the maternal serum and the allantoic fluid comes from the amniotic fluid by the intervention of the fetus.

Meschia, Battaglia and Barron (44) determined the osmotic pressure of the fetal and maternal serum of sheep and goats by freezing point depression techniques at various stages of gestation. They found that total osmotic pressure of the fetal plasma was equal to or slightly below that of the maternal plasma. They also demonstrated that any manipulation that interfered with the fetal side of the placental circulation invariably produced a rise in the total osmotic pressure of the fetal plasma. This suggests a reason for the early reports that osmotic gradients exist from the fetus to mother.

Alexander, Nixon, Widdas, and Wohlzogen (2) determined the composition of the fetal fluids in sheep and made comparisons of the concentrations of the constituents present with those in the maternal and fetal plasma and the maternal and fetal urine, through the fetal ages of 45 to 142 days. They observed no difference in osmotic pressure of the fetal and maternal plasma at the different stages of gestation studied. Creatinine concentration in the amniotic fluid was found to exceed the fetal plasma concentration but was less than that of the fetal urine. The creatinine level of the allantoic fluid was higher than that of

the fetal urine and the former positively correlated with
fetal age. This would appear to lend support to the theory
that the urachus drains the fetal urine to the allantoic sac.
No consistent trends were observed in the sodium concentration
of either the amniotic or allantoic fluids which had mean values
of 114 mEq/l. with a range of 86-146 mEq/l. and 70 mEq/l. with
a range of 32-131 mEq/l. respectively. Amniotic potassium
concentration apparently decreased with increasing fetal age
while the converse was observed in the allantoic fluid. This is
in accord with the findings of Malan et al (41). The range of
potassium concentrations for the amniotic fluid was 4.7 to 30
mEq/l. and for the allantoic fluid it was 1.0 to 33.0 mEq/l.
The mean fetal and maternal plasma sodium concentrations were
152 and 160 mEq/l. respectively, while the potassium values were
6.7 and 6.0 mEq/l. respectively. The authors concluded that the
probable source of the allantoic fluid is the fetal urine. The
amniotic fluid is probably derived from the fetal and maternal
plasma with fetal urine admixture in the latter part of gestation.
The fetus may play an active role in the modification of the
amniotic fluid by swallowing. It may be noted that these workers
observed a sodium concentration gradient from mother to fetus as
was observed by Phillips and Sundaram (48).

Davies (12) conducted experiments with sheep embryos of
about 15-18 days of age and found 2-5 mls. of allantoic fluid was
present and there was no collectable amniotic fluid present. The
sodium and potassium concentrations in the allantoic fluid were

95 and 8.5 mEq/l. respectively.

Flexner and Gellhorn (22) studied the comparative physiology of placental transfer in species of the various placental types. From these studies the authors concluded that those placentae which morphologically appear less permeable are in fact less permeable when tested physiologically. This was in general agreement with Grosser's classification of placentation. Flexner and Gellhorn (22) also determined that the placental permeability varies with the stage of gestation. In all species studied they found that the transfer rate of sodium per unit weight of placenta increased through gestation to a maximum just prior to term. There was a sharp drop as term approached. This was interpreted to mean that increased transfer rates occurred to accommodate the increased growth of the fetus and that the fall of the transfer rate as term approached was due to senile changes in the placenta.

Flexner, Cowie, Hellman, Wilde, and Vosburgh (21) observed the same relationship of an increase in the transfer rate of sodium with increasing age of the human fetus. The amount of sodium presented to the fetus was in excess of the amount incorporated in the fetal tissue; the ratio of these amounts being termed the "safety factor".

Hellman, Flexner, Wilde, Vosburgh, and Proctor (31) conducted a similar study using deuterium oxide to determine the permeability of the human placenta to water and to see if there was a similar change in the transfer rate with gesta-

tion age and also if excess water was presented to the fetus. It was observed that a curve similar to that for sodium was obtained for placental permeability of water. There was a five-fold increase in the permeability from the 14th week of gestation to the peak at about 35 weeks. Following the peak there was a sharp decline. There was also a large "safety factor". The authors concluded that variation in the permeability of the placenta to water was related to physical changes in the placental tissue namely thinning of the walls of the villi together with increased area of placental exchange. The sharp drop in permeability at term being due to placental senility.

Vosburgh, Flexner, Cowie, Hellman, Proctor, and Wilde (64) used tracer analysis to determine the renewal time of sodium and water in the amniotic fluid of humans. It was determined that the water of the amniotic fluid was renewed once every 2.9 hours and that sodium was renewed once in 14.5 hours.

In extending this work Plentl and Hutchinson (54) considered the experimental material as a two compartment closed system. The compartments were 1) the water of the mother and fetus and 2) the amniotic water pool. Any fetal intervention was ignored. They determined that the transfer of water to the amniotic pool was about 600 mls. per hour which agrees approximately with the estimations of Vosburgh et al (64). Gray, Neslen, and Plentl (28) extended the model to a three compartment closed system including in their analysis the

fetus as a separate compartment. On the basis of experiments with hydrodynamic models and on experiments in pregnant women, it was concluded that at least 25% and probably more than 50% of the water transferred between the amniotic fluid and the mother is accomplished through the intermedium of the fetus.

Plentl (51) continued with this line of investigation attempting to demonstrate in vitro, water exchange across the umbilical cord. Fresh human umbilical cords were perfused with heparinized blood while immersed in an artificial amniotic fluid. Tritiated water was added either to the perfusion fluid or the surrounding bath and the change in specific tritium activity was determined as a function of time. Rates of exchange of water between the two compartments were calculated to be 40.5 and 50.5 mls. of water per hour. The author concluded that the umbilical vessel plays a significant role in the water exchange of the amniotic fluid.

Using tracer analysis, Neslen, Hunter, and Plentl (47) in humans, simultaneously determined the rates of exchange of sodium, potassium and water between the amniotic fluid and the maternal system. Using two isotopes of sodium, the rate of exchange of sodium to and from the amniotic fluid was found to be equal, indicating a steady state system for sodium. The amount of sodium exchanged per minute was about 35 times greater than that of potassium. The ratio of these exchange rates is about the same as their concentration ratio in the amniotic fluid. The exchange rate for water was about five times greater than that for sodium and potassium. The authors concluded that

water and electrolytes of the amniotic fluid are in dynamic
equilibrium with maternal plasma each exchanging at its own
characteristic rate.

Plentl (50) and Hutchinson, Hunter, Neslen, and Plentl
(33) expanded this study to include a greater number of experi-
mental subjects. Plentl (50) noted that the amniotic fluid
constituent exchange rates are independent of the amniotic fluid
volume and proposed that the system can be represented by a cate-
nary three compartment system in which the end compartments ex-
change. Hutchinson et al (33) state that the supposition of the
amniotic fluid being a transudate or an ultrafiltrate of plasma
is based upon the similarity of the freezing point depressions
and electrolyte concentrations of plasma and amniotic fluid.
If such were the case, then the water, sodium, and potassium
should appear en bloc and be removed in the same manner. Having
established that water is transferred at a rate of about 26 mols.
per hour, this amount of solvent would carry with it about 68 mEq
Na and 1.9 mEq K. However, the experimentally determined transfer
rates of these electrolytes are about one-fifth that amount; a dif-
ference which was beyond the limits of experimental error. Water
and electrolytes are therefore exchanged by specific independent
mechanisms at their own characteristic rates. However, the average
exchange rates of 26 mols. of water per hour, 12 mEq Na per hour
and 0.6 mEq K per hour, specifies only the amount of these substan-
ces leaving and entering the amniotic fluid per hour, but gives no
information as to the sites of this exchange.

Villee (63) discussed the origin of the amniotic fluid.
Infants born with renal agenesis have no amniotic fluid (oligohydramnios),

while in contrast atresia of the oesoghagus is almost always
associated with excess amniotic fluid (hydramnios), which
suggests that under normal conditions the fetus swallows
amniotic fluid and excretes it through the kidneys. Wright
and Nixon (69) demonstrated that amniotic fluid may be absorbed
from all segments of the fetal gut, which tends to support the
theory presented by Villee (63). However, Plentl and Gray (53)
point out that if the rate of transfer of 300 mls. per hour
of water from the mother to the amniotic fluid is correct, then
a similar amount would have to be removed. At this rate degluti-
tion cannot be regarded as a primary route of removal. These
workers state that fetal micturition and deglutition play, at
best, a very subordinate role in the physiology and physio-
pathology of the amniotic fluid. Plentl and Gray (53) alterna-
tively offer the possibility that the amniotic epithelium has
secretory characteristics; however, they felt that not sufficient-
ly large quantities of fluid could be produced by these cells. How-
ever, the steady increase in amniotic fluid volume during the latter
half of human pregnancy occurs at a time when the amniotic cells
degenerate (53), which reduces the credibility of this hypothesis.

The bucal mucosa, lungs and salivary glands have been
proposed by Reynolds (55) as a possible source of the amniotic
fluid, but this view was based on acute experiments in which
the disturbance to the fetus was great. The fluid found in these
cavities may have been taken in by respiratory movements rather
than occurring by active secretion of the epithelial linings.
Liley (40) interprets these findings similarly, suggesting in

fact that the larynx _in utero_ may act as a sphincter protect-
ing the fetal lung from amniotic debris. However, Adams,
Moss, and Fagan (1) present strong evidence for active secre-
tion of tracheal fluid in fetal lambs near term. The fluid
collected by cannula at an average rate of 1-2 mls. per
minute was higher in sodium and chloride but lower in potassium,
protein, sugar, and urea than the amniotic fluid. Except for
the protein level the tracheal fluid was similar in composition
to the fetal blood.

Davies (12) demonstrated increasing concentrations of
urea, creatinine, potassium and calcium, and dilution of sodium
and chloride with increasing gestation age in the allantoic
fluid of the rabbit. The allantoic fluid of the pig also tends
to concentrate both urea and creatinine with increasing gestation
age (63). These findings tend to support the theory that the
allantoic fluid is of fetal kidney origin. In fact Barrow as
cited by Davies (12) speculates on the possibility of the allan-
toic sac being an extended part of the fetal kidney.

It is evident from the literature reviewed that there are
many factors that may contribute to the production and regulation
of the amniotic fluid. Designating any one factor as the major
source appears impossible.

The nature of the allantoic fluid suggests that fetal
urine is the principle source, but the discovery of the allantoic
fluid predating the amniotic fluid early in embryonic life (12),
leaves the origin of the allantoic fluid in doubt as well.

EXPERIMENTAL PROCEDURE

Experimental Animals

In order to study the sodium transfer rates among
the maternal and fetal sodium compartments twenty-five cross-
bred ewes, either Dorset Horn x Suffolk or Dorset Horn x
Corriedale, were purchased from a local producer. All the ewes
had lambed in the spring and ranged in age from 2-3 years.

Breeding of Animals

In an attempt to allot the caesarian operation dates
wherein operations would be performed at the same stage of
gestation (135 days) it was decided to attempt to control
estrus by feeding an orally active progestational compound,
6 - Chloro - 6 Dehydro - 17 Alpha Acetoxyprogesterone (CAP)
in lactose, after the method of Wagner (65). The treatment
was administered by placing the capsule containing CAP into
the region of the pharynx with the aid of a curved bowel clamp.
Treatment was given prior to the morning feeding; the procedure
taking no longer than a few minutes.

The ewes were allotted into five groups of five. The
first three groups were treated as anestrus ewes and fed CAP at
a level of 1 mg. per day for 16 days followed 36 hours after
the last CAP feeding by a subcutaneous injection of 1000 I.U.
of Pregnant Mare Serum (PMS). The animals were then placed
in a pen with an aproned Suffolk ram fitted with a marking

harness, and observed several times a day in order to deter-
mine the onset of estrus. The fourth group had exhibited
one heat period prior to being fed the CAP and were therefore
treated as cycling ewes; treatment being the same as the
first three groups except that no PMS was administered. The
fifth group of ewes had exhibited more than one heat period
and were therefore allowed to cycle normally and were permitted
to run with the aproned ram. When the aproned ram marked an
animal, the ewe was placed in a separate pen with a breeding
ram where coitus was observed. Conception was confirmed by
the bred ewes not returning to estrus within 30 days.

Diet

Ewes were group-fed 2 pounds of alfalfa-brome hay per
day prior to and during the breeding period. "Flushing" was
not attempted for it was desirable that only single lambs be
obtained. Subsequent to breeding the ewes were given a diet
consisting of:

```
        Barley-oat mixture (60:40) ....................1 lb./head/day.
        Brome-alfalfa hay ...........................free choice
        Mineral mix
            bone meal              )
            limestone              (equal parts ........ free choice
            cobalt-iodised salt    )
```

During the period of sodium depletion the salt mixture was with-
drawn and replaced with a potassium salt mixture (equal parts
potassium citrate, potassium acetate, and potassium bicarbonate)

that was supplied in the grain ration at a rate of 200 mEq K/head/day. Potassium at this level does not significantly change the normal course of sodium depletion by parotid drainage nor does it affect the parotid response to depletion (13). This procedure was followed in order to obtain sodium depletion per se without concomitant potassium depletion. Water was allowed ad libitum during breeding, gestation, and during the depletion period.

Depletion Procedure

Polyethylene tubing of I.D. ₀045", O.D. .062" was used for draining parotid saliva. Approximately 30 minutes prior to inserting the catheter the animal was given 50 mg. acepromazine malate (Atravet) intravenously into the jugular vein. The animal was then placed on its rump and held by an assistant. The right cheek area shaved and washed. A laryngoscope was passed along the buccal side of the cheek and the opening of the parotid duct located. With a wire stilette inserted in the polyethylene catheter of approximately 8" in length, the tubing was passed into the duct, withdrawing the stilette as the tubing was inserted in the duct. With the catheter in place, a 12 ga. hypodermic needle was passed through the cheek so as to emerge inside the mouth about one-quarter of an inch anterior to the opening of the parotid duct. The free end of the catheter was then passed through the lumen of the needle, brought outside, and the needle withdrawn. The catheter was fixed to the cheek at its point of

emergence by tying it to a superficial skin suture. Part
of the tube was snipped off so as to leave approximately 2"
projecting from the cheek. A free flow was established and
the saliva effectively drained; seven days being allowed
for depletion.

Preparation of Isotope and Detection Equipment.

Two batches of Na^{22} in saline of 1 mc. each were
obtained from The Radiochemical Center. For each shipment,
duplicate standards of .02 μc. in a volume of one ml. were
kept as counting standards. A solution containing 20 μc.
Na^{22} per ml. was made for injections; the standard injection
being 5 mls. delivered in an accurately calibrated glass syringe.
All ewes received 100 μc. Na^{22} except ewes DS-27 and DS-29, which
received approximately 35 and 50 μc. Na^{22} respectively. All
samples were counted in duplicate to obtain a minimum of 5000
counts. The scintillation counter consisted of a well-type
gamma crystal with a photomultiplier tube and a Picker Scaler,
model 600-010. Lead shielding was utilized to reduce the back-
ground count rate. To all samples counted, background corrections
were applied. It was deemed expedient not to correct for isotope
decay, the error here incurred was calculated to be well within
the experimental error. The operating potential of 775 volts
was selected by maximizing the "Figure of Merit" calculated as
(sample count rate)^2/(background count rate). Counter efficien-
cies calculated on the two shipments of isotope received were

approximately 27% and 32% respectively. All samples were
counted in a volume of 1 ml. in standard 100 x 13 mm. counting
tubes to preserve geometry.

Radiosodium Injection, Caesarian Section, and Sample Collection

Twenty-four or 36 hours prior to the caesarian section,
the ewes were brought from the barn to the surgery area and
placed in a metabolism crate. The neck area was shaved to faci-
litate sampling the jugular blood. A sterile urinary catheter
(14 Fr.,5 cc. bulb) was placed in the bladder and urine collect-
ed subsequent to the Na^{22} injection. Separate total urine
collectionswere made from injection to the time of the caesarian
section and during the test period. Five mls. (100 μc.) of
prepared Na^{22} solution were drawn into the calibrated syringe
and injected into the jugular vein of the ewe, care being
taken to rinse the syringe with blood several times before with-
drawing the needle. The time of injection was recorded. On the
day of the caesarian section, about two hours prior to the opera-
tion the first maternal blood sample was taken. Later samples
were taken hourly until the completion of the operation. One-
half hour prior to the operation 50 mg. of acepromazine malate
(Atravet) was given to the ewe to tranquilize the animal. The
ewe was then placed on it's back on the operating table and the
abdominal region was prepared for surgery. Xylocaine hydro-
chloride 2% with epinephrine 1:100,000 was used as a local
anesthetic. The uterus was exposed through a mid-line incision

along the linea alba from just anterior to the mammary gland to the umbilicus. A small incision (3/4-1") was made in the uterus at the anterior end of the pregnant horn and the allanto-chorionic membranes exposed. A fetal blood sample was drawn into a dry heparinized syringe from a fetal vessel of a cotolydon near the incision line, via a 22 gauge needle. Subsequent to sampling the whole cotolydon was ligated to prevent blood loss and the uterine incision sutured. At this time fetal fluid samples were collected by puncture through the uterine wall. The amniotic and allantoic fluids were distinguished at the time of sampling by color and viscosity. A 20 gauge needle and syringe was used to withdraw approximately 5 mls. of each fluid. When allantoic fluid was obtained, the needle was left in place and approximately 2 mls. of a 2% T-1824 dye solution was injected into the allantoic sac. This was done to check for physical leakage between compartments. The time of sampling was recorded (t_1). The uterus was returned to the abdominal cavity and the abdominal incision temporarily closed by means of interupted "through and through" relaxing sutures and a continuous skin suture. The animal was then returned to the metabolism crate. The second urine collection was started at this time. The whole procedure generally took no longer than one-half hour. Approximately 4 hours later (t_2) with no further anesthetic required, the animal was again placed on it's back, the incision reopened and the uterus this time being completely delivered onto the sterile drapes on the

abdomen. A fresh incision was made in the uterus approxima-
tely 3" in length and the allanto-chorionic membranes exposed.
The allantoic fluid was collected in a tray and the amniotic
sac with the fetus inside was lowered intact into another tray.
The second samples of allantoic and amniotic fluid were collect-
ed without contamination. At this time dye stains were looked
for in the amniotic fluid, none ever being observed. The umbilical
cord was then exposed and the second fetal blood sample was obtained
in a heparinized syringe. The umbilical cord was stretched to
facilitate breakage at the natural breaking point. No blood loss
occurred using this technique. The uterus was sutured and replaced
in the abdominal cavity and the abdomen closed. The amniotic and
allantoic fluid volumes were measured and the fetus weighed. The
fetus was then blotted dry and re-weighed; the difference in
weight being added to the amniotic fluid volume without correction
being made for specific gravity. All fluid samples and plasmas
were frozen subsequent to counting the radioactivity. Fetal
adrenal weights were taken on the pair of adrenals within 24
hours after delivery. The fetus was then frozen for subsequent
Na and K determination.

Analytical Methods

Plasma was separated by centrifuging the heparinized
blood in test tubes at 2500 r.p.m. for 20 minutes.

Feces radioactivity was estimated by macerating a
weighed amount of feces in a known volume of water for a minimum
of 96 hours, at which time duplicate one ml. samples of the
supernatant fluid were counted.

For the analysis of whole fetal tissue the whole
frozen fetus was passed through a Hobart grinder several
times, first using a 1/4 inch die then a 1/8" die in sub-
sequent runs, to obtain an homogenous mixture. Representa-
tive 5-10 gm. samples were placed in 100 ml. Kjeldahl flasks
and partially digested with 20 ml. of concentrated nitric
acid after which 5 mls. of perchloric acid (70%) was added and
the heat increased, care being taken to add small amounts of
concentrated nitric acid until digestion was complete. The
digest was cooled, filtered through Whatman No.40 filter
paper and made up to 100 ml. volume with de-ionized water.
Radioactive counts were made directly on this solution.

Maternal plasma, fetal plasma, maternal urine, amniotic
and allantoic fluid samples, were measured for radioactivity
by pipetting 1 ml. into the counting tubes and placing in the
scintillation counter.

Saliva, maternal plasma, fetal plasma, amniotic and
allantoic fluid, and whole minced fetal tissue digest, were
analyzed for total sodium and potassium by direct intensity
flame photometry. The method used was that described by the
manufacturer (34). A wave length of 589 mμ was used for sodium
and 404 mμ for potassium determinations. The bracketing method
was used for potassium determinations while for sodium, a
standard curve of percent transmittance over known sodium
concentrations was established and unknown samples were
interpolated from this graph. Sodium chloride was added to
all potassium working standards so that the sodium concentra-
tion in the standards was similar to that found in the sample

dilutions. In the case of plasma, an artificial plasma was used to make the working standard for potassium analysis. The artificial plasma consisted of, in g/l., NaCl, 8.072; $CaCl_2 \cdot 2H_2O$, 0.370; $MgSO_4 \cdot 7H_2O$, 0.250 in de-ionized water (32).

Total maternal exchangeable sodium was calculated by the following formula:

$$Ex.Na = \frac{Na_r \quad x \; Na_{pl}}{R_{pl} \quad x \; 1000}$$

where $Ex.Na$ =   total exchangeable sodium, in mEq

         Na_r =   counts remaining in the maternal system, in cpm

         Na_{pl} = sodium concentration in maternal plasma, in mEq/l.

         R_{pl} = mean count rate of maternal plasma, in cpm/ml.

The total counts remaining in the maternal system was calculated by correcting the total counts injected for the radioactivity excreted in the urine and feces and that accumulated by the conceptus during the initial equilibration period from the time of injection (t_0) to the time of the first fetal samples (t_1). This period $(t_0$ to $t_1)$ was approximately of 24 hours duration for control animals and 36 hours for the sodium depleted ewes. The longer equilibration time was thought necessary for the depleted group for the rumen sodium pool of these animals was probably reduced; thus the equilibration time was likely to be longer.

Total exchangeable fetal sodium was estimated by

determining the total body sodium of the fetal lamb. Munro
et al (46) suggested that young bones have a more dynamic
sodium pool which equilibrates more rapidly with the extra-
cellular sodium pool than that of older animals. Pitts
(49) states that the total body store of sodium approximates
the exchangeable sodium pool except for that which is occluded
in the compact bone. In the fetal lamb there is little compact
bone; therefore total fetal body sodium would not include a
great error as an estimate of total exchangeable fetal sodium.

Total sodium of the amniotic and allantoic fluid
compartments were considered as the respective exchangeable
sodium pools.

Statistical Methods and Tracer Analysis

Unpaired t-tests as outlined by Steel and Torrie
(59) were used on the maternal and fetal data. Maternal
plasma specific activities were tested with simple linear
regression analysis and regression equations calculated
(59).

The theory of tracer kinetics for multicompartment
systems as outlined by Sheppard (58) was applied to the
data. The model proposed was that of a closed unconstrained
four compartment system in a steady state. The four compart-
ments were: the maternal exchangeable sodium pool, the fetal
exchangeable sodium pool, the amniotic fluid sodium pool, and
the allantoic fluid sodium pool. Multiple experiments were
conducted in order to obtain the necessary number of equations

to solve for the proposed twelve transfer rates of sodium.
The final set of equations, using the summation notation,
was then:

$$\frac{d(^{r}R_i)}{dt} = \sum_{\substack{j \\ j \neq i}} p_{ij} \; ^{r}a_j - \; ^{r}a_i \sum_{\substack{j \\ j \neq i}} p_{ji} \ldots\ldots\ldots (1)$$

where $\dfrac{d(^{r}R_i)}{dt}$ = the rate of change of total radioactivity
in compartment i of experiment r, in cpm
per hour.

$^{r}a_i$ = the specific activity in compartment i
during experiment r in cpm/mEq Na.

and p_{ij} = The transfer rate of sodium (rho) from
compartment j to compartment i in mEq/
hour.

The expansion of this general equation provides the twelve
equations in the 12 unknown rho's (p).

The function $dR_{i/dt}$ was calculated from the relation:

$$dR_{i/dt} = S_i \cdot da_{i/dt} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

where $dR_{i/dt}$ = rate of change of total radioactivity in
compartment i, in cpm/hr.

S_i = total amount of traced element (sodium)
in compartment i, in mEq Na.

and $da_{i/dt}$ = rate of exchange of specific activity

in compartment i, in cpm/mEqNa/hr.

The function $da_{i/dt}$ was calculated from:

$$\frac{a_{t_2} - a_{t_1}}{t} = da_{i/dt} \cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots (3)$$

where a_{t_1} and a_{t_2} = specific activities at time t_1

and t_2 respectively measured in

cpm/mEq Na.

To exemplify, the expansion of equation 1 for

compartment 1 of the third experiment would be:

$$^3a_2p_{12} + {}^3a_3p_{13} + {}^3a_4p_{14} - {}^3a_1p_{21} - {}^3a_1p_{31} - {}^3a_1p_{41}$$

$$= \frac{d^3R_1}{dt} .$$

Likewise, compartment 2 of the fourth experiment would be:

$$^4a_1p_{21} + {}^4a_4p_{23} + {}^4a_4p_{24} - {}^4a_2p_{12} - {}^4a_2p_{32} - {}^4a_2p_{42} = \frac{d^4R_2}{dt} .$$

These equations were further expanded to twelve unknown terms

and rearranged to obtain the desired matrix by inserting zero

values where appropriate. The final matrix was a
square array of unknown p's arranged in twelve rows
and columns. The resulting matrix was solved for the
twelve unknown p's using an IBM 1620 computer with a
general program for the solution of homogenous and
non-homogenous simultaneous equations.

RESULTS

Of the 15 ewes treated as anestrus ewes 14 demonstrated overt estrus within 3.5 days of the PMS treatment; the mean time was 2.32 days. Twelve of the 14 ewes bred at first estrus conceived. Three out of the five ewes in the fourth group demonstrated overt estrus within seven days of the last CAP feeding; the mean time was 6 days. All three conceived at first estrus. All five of the ewes in the fifth group conceived at first breeding which occurred within a 27 day period.

Sodium depleted ewes DC-12, DS-54, and DS-58 aborted dead fetuses prior to caesarian section. Examination revealed no reason for the fetal loss.

Ewe DS-32 was sampled after only 6.5 hours equilibration. The data from this ewe therefore was not used for compartment analysis.

During the seven days of sodium depletion, with the exception of only a few animals, the ewes continued to eat normally. Those ewes that would not eat the complete grain ration were drenched with a $KHCO_3$ solution to maintain the supplementary potassium intake. It was not apparent that water intake was reduced, although water consumption was not measured.

Maternal Changes

The results of the maternal data are summarized in

Table I.

Some ewes appeared to be affected more severely
than others over the seven day depletion period which
was indicated by the finding that some ewes had lower
salivary sodium levels after the depletion period. In
all cases however, sodium depletion produced lowered
Na:K ratios with a treatment mean and standard error of 1.998 $\pm$
2.996.

Although no measurements were made of the daily volume
of parotid saliva, a decreased rate of saliva flow was sug-
gested by the increased length of time required to collect
the necessary volume of saliva from the "more severely
depleted" ewes.

Sodium depletion caused a significant decrease
($P<.05$) in the maternal plasma sodium concentration. The
treatment means for the control and depleted groups were
149.0 and 137.5 mEq Na/l. respectively. Plasma potassium
concentration was shown to have a slight, although not
significant increase from 4.45 mEq/l. for the control group,
to 4.84 mEq/l. for the depleted group.

The means of maternal exchangeable sodium pool
size of the control and depleted ewes were 2214.5 and
1799.34 mEq Na respectively. While this difference was
not significant at 5% it was significant at the 10% level.
However, no statistical significance was observed when
this parameter was expressed as a function of maternal body

TABLE I.   Summary of maternal data
showing means and standard errors of
control and depleted groups.

	Control	Depleted
Saliva Na:K Ratio	–	1.998 ± 2.996 (5)
Plasma Sodium (mEq/l.)	149.0 ± 2.78 (7)	137.48 ± 3.35 (5) ✱
Plasma Potassium (mEq/l.)	4.45 ± 0.30 (7)	4.84 ± 0.25 (5)
Exchangeable Sodium (mEq)	2214.5 ± 65.16 (4)	1799.34 ± 172.65 (5)
Exchangeable Sodium (mEq/100 gm. BW)	3.38 ± 0.14 (4)	3.11 ± 0.28 (4)
Body Weight (Kgm.)	65.07 ± 2.55 (6)	60.61 ± 2.15 (4)

✱ $P < .05$

Figures in parentheses indicate number of observations

weight. Expressed in this way the mean maternal
exchangeable sodium of the control and depleted ewes were
3.38 and 3.11 mEq/100 gm. body weight respectively.

Maternal body weight was not observed as a para-
meter through the period of sodium depletion. However,
it was observed that the depleted group had a smaller mean
body weight, though not statistically significant, than
the control group. The mean difference was 4.46 Kgm.

Fetal Changes

The fetal data for both the control and depleted
groups is summarized in Table II. All fetuses were viable
at the termination of each caesarian.

There was no significant difference in the mean
fetal plasma sodium levels between the control and
depleted groups, although the depleted group was shown to
have a lower mean level. The respective means were 144.42
and 139.15 mEq Na/l. Fetal plasma potassium concentration
was not statistically different between the control and
depleted groups; the respective means and standard errors
were 5.73 $\pm$ 0.87 and 7.02 $\pm$ 3.31 mEq K/l.

Whole minced fetal tissue dry matter was signifi-
cantly different ($p < .01$) between treatment groups. The
means percent dry matter for the control and depleted

TABLE II.   Summary of fetal data
showing means and standard errors of
the control and depleted groups.

	Control			Depleted			
lasma odium (mEq/l.)	144.42	± 8.27	(9)	139.15	± 11.43	(5)	
lasma otassium (mEq/l.)	5.73	± 0.87	(9)	7.02	± 3.31	(5)	
hole Minced Tissue (mEq/100 gm.)	8.60	± 0.11	(7)	8.31	± 0.26	(5)	
hole Minced Tissue (mEq/100 gm.)	4.44	± 0.07	(7)	4.43	± 0.11	(5)	
hole Minced Tissue ry Matter (%)	21.27	± 0.29	(9)	23.69	± 0.38	(5)	**
etal Weight (gms.) [a]	4186.6	± 255.8	(5)	4021.4	± 125.6	(5)	
xchangeable Na (mEq) [a]	355.26	± 25.08	(5)	333.20	± 9.26	(5)	
drenal Weight (gms.)	0.483	± 0.032	(7)	0.561	± 0.022	(5)	
drenal Weight (gms./100 gms. FW)	0.125	± 0.030	(7)	0.138	± 0.015	(5)	
etal Age (days)	135.6	± 1.4	(6)	137.6	± 1.4	(5)	
miotic odium (mEq/l.)	119.14	± 32.13	(9)	96.85	± 25.35	(5)	
miotic otassium (mEq/l.)	6.42	± 3.00	(9)	9.16	± 6.40	(5)	
llantoic Sodium (mEq/l.)	73.75	± 18.31	(9)	47.05	± 21.73	(5)	*

TABLE II. — continued

	Control				Depleted			
llantoic tassium Eq/1.)	37.61	±	18.87	(9)	53.69	±	26.64	(5)
miotic lume (mls) a	1030.0	±	67.54	(4)	1781.2	±	193.66	(5) ⚹
llantoic lume (mls) a	726.25	±	129.72	(4)	839.4	±	173.22	(5)

a, Twin Data Omitted; ⚹ P<.05 ;   ⚹⚹ P<.01
Figures in parentheses indicate number of observations.

groups were 21.27 and 23.69% respectively.

The mean fetal weights of the control and depleted
groups were 4189.6 and 4021.4 gms. respectively. The
difference between means was not significant.

Whole minced fetal tissue sodium and consequent-
ly the total fetal exchangeable sodium was not significant-
ly different between treatment groups. The means and
standard errors of total exchangeable sodium of the control
and depleted groups were 355.26 $\pm$ 25.08 and 333.20 $\pm$ 9.26
mEq Na respectively. Expressed as a function of fetal
body weight the respective means and standard errors were
8.60 $\pm$ 0.11 and 8.31 $\pm$ 0.26 mEq Na/100 gms. fetal body
weight. Expressed either as an absolute value or as a
function of fetal weight exchangeable fetal sodium was
not significantly affected by treatment. Similarly, fetal
tissue potassium levels were not significantly affected
due to sodium depletion of the mother.

Fetal adrenal weights were not significantly
different between treatment groups even though the
depleted group had a larger adrenal weight. The mean
adrenal weights of the control and depleted groups were
0.125 and 0.138 gms./100 gms. fetal body weight respecti-
vely.

Amniotic fluid sodium concentrations for the

control and depleted groups were not significantly
different; the respective means and standard errors
being 119.14 $\pm$ 32.13 and 96.85 $\pm$ 25.35 mEq/l. Amniotic potas-
sium levels also were not significantly different; 6.42 $\pm$
3.00 and 9.16 $\pm$ 6.40 mEq K/l. respectively.

Allantoic fluid sodium concentrations were signifi-
cantly different (P<.05) between control and depleted groups;
the respective means and standard errors being 73.75 $\pm$ 18.31
and 47.05 $\pm$ 21.73 mEq Na/l. No significant difference was
observed for allantoic potassium concentration between groups,
the means and standard errors being 37.61 $\pm$ 18.87 and 53.69 $\pm$
26.64 mEq K/l. respectively for the control and depleted
groups.

Amniotic fluid volume of the depleted ewes was signi-
ficantly greater (P<.05) than that of the control animals.
The means and standard errors for the amniotic volume of
the control and depleted groups were 1030.0 $\pm$ 67.54 and 1781.2
$\pm$ 193.66 mls. respectively. Allantoic fluid volume was not
significantly different between groups; the respective means
and standard errors were 726.25 $\pm$ 129.72 and 839.40 $\pm$ 173.22
mls.

Tracer Analysis

The data showing compartment sodium specific

activities with time for the control and sodium-
depleted ewes are given in Tables XX and XXI respecti-
vely. The data of ewes DC-2, DS-5, DS-11, and DS-25 of
the control group and ewes DC-17, DS-27, DS-29, and DS-37
of the sodium-depleted group were utilized as outlined in
the experimental procedure for the calculation of the
sodium transfer rates among compartments after equating
the rate of change of total radioactivity of the matern-
al compartment to the sum of the rates of change of total
radioactivity in the fetal compartments. The results
are given in Table III.

TABLE III. Transfer rates of sodium
(mEq/hr.) between compartments using
four compartmental analysis for control
and depleted groups.

Transfer Rate	Control	Depleted
P_{21}a	318.3	0.6
P_{12}	615.8	-13.4
P_{31}	-90.8	5.5
P_{13}	647.9	11.6
P_{41}	191.9	0.0
P_{14}	373.7	63.4
P_{32}	132.7	-2.4
P_{23}	-511.2	-0.3
P_{42}	-287.2	0.9
P_{24}	-317.2	-72.2
P_{43}	-306.5	-0.1
P_{34}	47.9	11.5

a, P_{21} = Transfer rate from compartment 1 to compartment 2,

P_{12} = etc.

where   compartment 1 = the maternal exchangeable sodium pool
compartment 2 = the fetal exchangeable sodium pool
compartment 3 = the amniotic fluid sodium pool
compartment 4 = the allantoic fluid sodium pool

DISCUSSION

Effects on the Ewe

During the period of sodium depletion there was
no apparent loss of appetite. This was unlike that
reported by Phillips and Sundaram (48), who observed
severe anorexia; however, their animals had an apparent
potassium depletion concomitant with sodium depletion and
it was more likely that the potassium deficit manifested
itself in the anorexia (16). Potassium depletion was
avoided in the present experiment by maintaining a potas-
sium supplement of 200 mEq/day over and above the potassium
present in the normal diet (14, 15, 16).

The sodium-depleted ewes were shown to have a lowered
salivary sodium to potassium concentration ratio which is
in accord with the findings of Denton (14, 15), who indicated
that the normal sheep parotid saliva Na:K ratio is about
18.0. Of the five depleted ewes studied, three had a saliva
Na:K concentration ratio of less than one, the fourth had
a value less than two, while the fifth (DS-27) was not
depleted to the same extent having a saliva Na:K ratio of
about eight at the end of the depletion period. Using this
phenomenon as a criterion for the extent of sodium depletion,
four of the five ewes could be considered well depleted while
the fifth was only moderately depleted.

The maternal plasma sodium changes due to sodium depletion reported in this experiment are in agreement with the findings of Phillips and Sundaram (48), in pregnant sheep and with Denton (16), in non-pregnant sheep. Sodium depletion caused a significant decrease ($P<.05$) in maternal plasma sodium; the mean decrease being 11.5 mEq Na/l. The mean maternal plasma potassium was observed to increase slightly, but this increase of 0.39 mEq K/l. was not significant. An increase in maternal plasma potassium was not observed by Phillips and Sundaram, possibly due to the potassium deficit that occurred during the depletion period. Denton (16) reported a mean increase of 1.2 mEq/l. in the plasma potassium concentration during sodium depletion with adequate potassium intake in non-pregnant sheep. This may indicate that the potassium supplement given in the present experiment (200 mEq K/day) was insufficient for gestating ewes in order to obtain an equivalent plasma potassium concentration increase to that reported by Denton (16) for non-pregnant sheep or that some difference exists between pregnant and non-pregnant animals in this regard.

The difference between the maternal exchangeable sodium pool size of the control and depleted groups was not statistically significant although the latter was 415 mEq smaller. This difference cannot be explained by the body weight differences because if the maternal exchangeable sodium is expressed as a function of body weight a smaller sodium pool is still

apparent for the sodium-depleted group although this difference also is not statistically significant.

Maternal body weight was not significantly affected by sodium depletion as it was in the experiments of Phillips and Sundaram (48) and Kirksey and Pike (35). It was thought that the potassium supplement given to the depleted ewes allowed the animals to maintain total osmotic balances and thus prevented the extracellular fluid volume from decreasing significantly.

Effects on the Fetus and Fetal Fluids

Sodium depletion had no appreciable effect on the fetal plasma sodium level. A decrease of 11.5 mEq Na/l. in the maternal plasma was associated with a mean decrease of only 5.3 mEq Na/l. in the fetal plasma which was not significant. This was in general agreement with Phillips and Sundaram (48) in pregnant ewes, and Kirksey et al (36) in pregnant rats. The latter reported a slight non-significant decrease in the fetal plasma sodium due to a deficit of sodium in the mother. Winkler, Theil, and Goetze (68) observed similar results in pregnant rats where sodium depletion was induced by peritoneal dialysis. A non-significant fetal plasma sodium decrease with a significant decrease of the maternal plasma sodium concentration due to sodium depletion, suggests that the fetal plasma sodium

maintains some degree of independence from the maternal
sodium level. This was also suggested by Phillips and
Sundaram (48) and Kirksey et al (36). A similar mecha-
nism for fetal potassium independence was suggested by
Stewart and Welt (61).

Fetal plasma potassium levels were not signifi-
cantly affected by sodium depletion. There is an appa-
rent increase in the mean value of plasma potassium
of the depleted group but this was mainly attributable
to the large plasma potassium level attained by the
fetuses of ewe Ds-10 (Table X). The plasma potassium
level of the fetus in this ewe was found to be 13.7
mEq/l., which accounts for the large standard error of
the group. No apparent reason could be found for this
high value but it may be noted that the fetal plasma
sodium level was quite low, which suggests that the
high potassium level was not an error in analysis.

The mean concentration gradient of plasma sodium,
which in normal ewes was found to exist from the mother
to the fetus, was reversed by sodium depletion of the
mother. This was also observed by Phillips and Sundaram
(48); Kirksey et al (36), and Alexander et al (2). Whether
this is a true electrochemical gradient is not apparent.
It may well be that changes in osmotic gradients occurred
to offset these sodium concentration gradients. Meschia
et al (44), in their osmotic pressure determinations of

maternal and fetal plasmas of sheep and goats, observed
that any manipulation interfering with the fetal side
of placental circulation invariably produced a rise in
the total osmotic pressure of the fetal plasma. During
the course of the present experiment fetal placental
circulation was interfered with to the extent of ligating
a placental cotolydon. The interference may well have
increased fetal osmotic pressure which in turn may counter-
act the sodium concentration gradient reported here and in
the earlier reports (36, 48).

Whole minced fetal tissue sodium and consequently
fetal exchangeable sodium was not significantly affected
by sodium depletion of the ewe. This is in general agree-
ment with Kalyansundaram (37) who observed no difference
in the fetal muscle sodium levels in response to sodium
depletion of the ewe. Expressed as a function of fetal
weight there is a small mean difference between groups
where the depleted group was shown to have the smaller
fetal exchangeable sodium pool. Stewart and Welt (61),
in their potassium depletion studies in pregnant rats,
observed an absolute reduction in the fetal potassium
pool due to potassium depletion of the mother. Although
the fetal sodium deficit reported here is not significant
it tends to conform to that reported for potassium by
Stewart and Welt (61).

It was thought that if the fetal sodium was independent of maternal influence then it may be possible that this was achieved through an independent fetal adrenal response and therefore a fetal adrenal weight change would be observed. However, there was no significant change in fetal adrenal weight due to sodium depletion of the mother. Nevertheless the difference in mean adrenal weights observed between groups may at least indicate that the fetal adrenals are functional as was indicated by Jones et al (11), with the fetal lamb and by Eguchi (20) in the fetal mouse. Possibly a more sensitive test involving histological techniques or hormonal assay would have given a better indication of this response.

Whole minced fetal tissue dry matter content of the depleted group was significantly greater than that of the control group thus indicating the possibility of some dehydration of the fetus. Although the mean fetal weight of the depleted group was 168.2 gms. less than that of the control group, the significantly different dry matter content of the two groups was not reflected in a significantly different fetal weight.

Sodium and potassium concentrations of the amniotic fluid were not significantly affected by sodium depletion of the ewe. The concentrations observed were in close

agreement with those of Phillips and Sundaram (48)
while Pike et al (36) observed similar results with
pregnant rats. However, in the study of Phillips
and Sundaram significantly lower amniotic sodium
concentrations were observed due to sodium depleti-
on of the mother, while in the present experiment
the difference was non-significant.

Considering the total amount of sodium and
potassium of the amniotic fluid of the depleted
group, it was found that both were increased over
those of the control group. This was not observed
by Phillips and Sundaram (48); in fact, the total
sodium of the amniotic fluid decreased in the de-
pleted group in their study.

Allantoic sodium concentration was signifi-
cantly decreased due to sodium depletion of the ewe.
Allantoic potassium on the other hand, was shown to
be increased, although not significantly, by sodium
depletion. This was similarly reflected in the total
sodium and total potassium of the allantoic fluid.
These findings are in disagreement with Phillips and
Sundaram (48) who observed no change in allantoic
sodium or potassium levels due to maternal sodium
depletion. A possible explanation would be that in
the present study potassium was probably more availa-

ble to the fetus than in the experiment by Phillips and
Sundaram where potassium depletion of the ewe occurred
concomitantly with sodium depletion. The more readily
available potassium would, under conditions of sodium
deficiency, allow the fetal kidney to excrete potassium
as the monovalent cation in place of the sodium ion.
The fact that this is most markedly seen in the allan-
toic fluid lends support to the theory that the fetal
urine is a source of the allantoic fluid and this pheno-
menon would then allow the allantoic sodium level to
decline to the extent that was observed. If this is
true, then it would refute the argument put forth by
Jacques as quoted by McDougall (43) that the urachus is
not patent in the later part of gestation.

Malan et al (41) in their studies of fetal fluids
of sheep observed the fetal fluid volumes to be nearly
equal at term. The average age of the animals studied
in this experiment was close to term or about 136 days.
At this advanced stage of gestation the volumes of the
two fetal fluids were nowhere near equal. The difference
between the fluid volumes was more marked in the depleted
ewes, where the amniotic fluid volume was 941 mls. larger
than the allantoic fluid volume, while for the control
group the amniotic fluid volume was 303 mls. larger than
the allantoic fluid volume. Comparing the fluid volumes

between groups, a significantly larger amniotic fluid
volume was observed for the depleted ewes while the
allantoic fluid volumes were not significantly diffe-
rent. The greater amniotic volume of the depleted
group could partially be explained due to the reduced
moisture content of these fetuses; assuming that the
water of the fetuses of the depleted ewes, in response
to the sodium deficit, was excreted to the amniotic
fluid. This theory, that the fetus reacts to sodium
depletion by excreting water in an attempt to maintain
homeostasis,was also suggested by Phillips and Sundaram
(48). However, in the present experiment, the difference
in dry matter content between the fetuses of the control
and depleted ewes was not large enough to fully explain
the 73% increase in amniotic fluid volume observed in the
depleted group. As mentioned, the total sodium and potas-
sium of the amniotic fluid were shown to be increased in the
depleted group. This observation in view of the increased
fetal dry matter content of the depleted group, would tend
to suggest that the increase in the amniotic fluid volume
was not totally derived from water excreted by the fetus.

The amniotic fluid was found to contain large
amounts of mucus material which was apparently derived from
the buccal area of the fetal lamb. When the fetuses were
allowed to drain by suspending from the hind legs, mucus

material continued to drain from the mouth for a
considerable period. If this fluid flowed in utero,
as was suggested by Reynolds (55) and Adams et al
(1) then it is possible that this material modifies
the amniotic fluid constituents. If this mucus
material is osmotically active then this alone could
have a considerable modifying effect on the osmotic
gradients between fluid compartments.

Tracer Kinetics

In an attempt to follow the mathematical analysis
of the tracer method suggested by Sheppard (58), three
basic assumptions were made. First, the model system as
proposed was in a steady state with respect to the traced
element, sodium, over the time of the experiment; second,
that each compartment was uniformly mixed with respect to
the labelled element at the time of sampling; and third,
that the labelled isotope of sodium behaves in exactly the
same manner as the non-labelled intrinsic sodium.

From pre-experimental evidence it was noted that
the radiosodium of the fetus did not reach equilibrium
with the radiosodium of the maternal system for several
days; in fact, the equilibrium point was never experimentally
determined. From the data of Tables XX and XXI

it was apparent that the radioactivity was nowhere near

equilibrium in the compartments after more than 40 hours.

It was, therefore, thought valid to estimate the rates

of change of sodium at a time after the maternal pool

had attained reasonable equilibration, yet before the

conceptus becomes equilibrated. In one animal (DS-32)

the rumen fluid was sampled after injecting the ewe with

radiosodium. It was found that the rumen count rate tended

to equilibrate with the maternal plasma count rate at about

nine hours post injection. Rumen specific activities were

not calculated on this animal. On the basis of this

animal it was thought that 24 to 36 hours would assure

equilibration of all the subcompartments of the maternal

sodium pool, excepting the conceptus.

The data recorded in Tables XX and XXI were seen

not to fulfill the requirement put forth by Sheppard (58)

that, in any closed system the total activity is constant;

that is, the rate of change of total activity in the initial-

ly labelled compartment should equal the negative of the sum

of the rates of change of radioactivity in all other compart-

ments. This condition was not satisfied in any of the expe-

rimental animals; therefore, the data could not be utilized

as suggested by Sheppard (58).

It is thought that considerable criticism could be

directed at the route of administration of the label; that

is, into the maternal compartment. Freedman, Grey,

Hutchinson, and Plentl (24) in their report of sodium

exchanges in the monkey, criticize the use of the maternal

system as the primary compartment due to its large size

and initial mixing, that makes it unsuitable as such, if

the experimental data are collected for the purpose of

calculating transfer rates. These workers, then, made no

attempt to use the maternal pool as the initial compartment

Likewise, in the present experiment the maternal system should

not have been used as the primary compartment. Furthermore,

by using the maternal system as the primary compartment

another difficulty was observed. The maternal exchangeable

pool size was several times larger than the total sodium

pools. Due to this large difference it was seen that a relati-

vely small change in specific activity of the large compart-

ment produced a very large change in the specific activity of

the smaller compartments. The large change of the specific

activity in the small compartment was easy enough to detect

with the detection equipment used, but accurately detecting

the small change in the maternal plasma specific activity was

not always possible. In some cases the changes observed in

the maternal sodium specific activity were not greater than the

counting error of the maternal sodium counts. This would tend

to limit the confidence ascribed to the maternal data. After

considering the criticism directed at the maternal pool as the

primary compartment and the error in observing the
rate of change of total radioactivity (dR_1/dt) in the
maternal compartment it was decided to equate the rate
of change of total radioactivity of the maternal compart-
ment to the sum of the rates of change of total radioacti-
vity in the fetal compartment; that is, fulfilling the
stipulation of a closed system. This done, the data were
then submitted to the IBM 1620 computer for solution and
the results obtained are listed in Table III. It is seen
from these results that the transfer rates are not compa-
tible with the theory of a steady state system. Negative
values of transfer rates were not acceptable for the pro-
posed model. By definition, the transfer rate must have
a value equal to or greater than zero. These results may
lead one into thinking that the proposed model was not
adequate. However, it is probably closer to the truth that
the data obtained was far too variable for this mathematical
analysis.

A normal ewe (DS-45) was fitted with a rumen cannula
prior to the radiosodium injection. Samples were taken of
the maternal blood and the rumen fluid for several hours post
injection. The specific activity of the rumen fluid was
observed to exceed the plasma sodium specific activity at
about six hours post injection and continue to increase
up to about 22 hours where it appeared to plateau. The

maternal plasma specific activity appeared to plateau
somewhat earlier at about 15 hours post injection. The
rumen specific activities were calculated using a rumen
sodium concentration of 78.0 mEq/l., which was determined
on a rumen fluid sample obtained by suction from the ventral
sac via the rumen cannula. This level of rumen sodium is some-
what lower than anticipated for a non-sodium-depleted sheep;
however, rumen dehydration was inadvertently superimposed on
this condition. Water had been withheld from the animal for
30 hours and numerous rumen fluid samples had been taken.
When the animal was released to the pen she immediately
drank a large amount of water. Why the rumen sodium pool
should appear to trap or accumulate the radioactivity is not
apparent. Perhaps the rumen sodium concentration was not
constant but was increasing due to dehydration. If this occurred
then rumen sodium specific activity would have been lower than
that actually calculated and possibly would have equilibrated
with the plasma specific activity. However, this was not tested
and it remains an uncertainly as to whether or not the radio-
sodium was truly in equilibrium in the maternal system over
the test period. These observations, to a large extent, sustain
the criticism of Freedman et al (24) that was directed at
using the maternal system as the primary compartment.

Further variability of the tracer data was seen in
Tables XX and XXI, most notedly in the parameter of the rate of
change of specific activity (d_a/dt). For example, da/dt for
the fetal compartment (compartment 2) of ewe DC-2, was the

largest of the compartments of this ewe; while in ewe

DS-5, compartment 3 (amniotic fluid) had the largest

da/dt; while in ewe DS-11, the da/dt of compartment 4

(allantoic fluid) was the largest. In other words

there was no consistent trend seen in the parameter

da/dt within any one group.

After considering the results and the data pre-

sented it was decided that the data was not suitable for

analysis as a four compartment system. The model was

then revised to a two compartment system and several vari-

ations of two compartmental analysis were attempted again

following a method outlined by Sheppard (58). The models

used are outlined in Appendix A along with the results

using these models. The results from all four models

are similar reflecting the similarity of their calcula-

tion. However, the results are of the same order of

magnitude as those reported by Freedman et al (24) for the

transfer rate of sodium from the amniotic fluid to the

maternal organism in both monkey and humans. Hutchinson

et al (33) and Neslen et al (47) reported similar results

in the human. In models 1, 3, and 4 of Appendix A, the

specific activity of compartment 2 has been calculated by

assuming that the subcompartments of the conceptus, which

we know to exist, are completely unconstrained with respect

to sodium, which is not quite true, for it was apparent in

the original data that the rates of equilibration of

sodium-22 were different for the three compartments of

the conceptus. These models (1,3, and 4) assign a mean weighted

specific activity which is assumed to be representative of

compartment 2. Sheppard (58) states that this method of

assuming inhomogeneity or incomplete mixing in a compartment

can be valid if sampled early following the administration

of the label, but as was the case in the present experiment,

the method becomes invalid. On the other hand, model 2, as

outlined in Appendix A, does not utilize a mean weighted

specific activity for compartment 2, but uses the specific

activity observed in the fetal plasma. Model 2 essentially,

attempts to measure the transfer rate of sodium between the

mother and fetus and to a large extent ignores the sodium

pool of the fetal fluids as part of compartment 2. Model 3

assumes that the amniotic and allantoic fluid sodium is derived

directly from the maternal system. Model 4 on the other hand,

assumes that the amniotic and allantoic fluid sodium is

derived via the fetus.

Examination of the data revealed that the fetal plasma

specific activity (Table XX and XXI) was always greater than

the whole minced fetal tissue specific activity (Table XIX).

The difference ranged from 5 to 20% with one fetus (DC-2)

having a 35% difference. This suggests that the fetal

plasma specific activity may be a poor estimate of the

exchangeable sodium of the fetal compartment.

At the outset of these experiments it was thought
that the tracer method may offer some insight as to the
source of the amniotic and allantoic fluids. The four
compartmental analysis attempted would possibly have
yielded some information on this matter. However, two
compartmental analysis, at best would only yield informa-
tion of the rates of sodium transfer between the mother and
the conceptus and not among the fetal compartments themselves.
It is thought that the four compartment model is the best
model for such a study but many refinements are obviously
needed to obtain suitable data for the mathematical ana-
lysis. A thorough study of sodium equilibration in the
ruminant animal is suggested. Likewise, the route of
injection should perhaps be changed if a similar study is
to be undertaken.

SUMMARY

1.   Five pregnant ewes at a late stage of gestation were depleted of sodium by unilaterally draining the parotid saliva. Seven pregnant ewes were maintained as control animals.

2.   After injecting the ewes with sodium-22, analysis of the data for sodium transfer rates among compartments in a closed, steady-state, unconstrained, four compartment system failed to yield rational results.

3.   Four models of a two compartment, unconstrained, closed, steady-state system were considered. Using this analysis transfer rates of sodium were calculated that were similar to those reported by workers for other species.

4.   The data indicated that sodium depletion caused: a statistically significant decrease in the maternal plasma sodium level; a significant increase in fetal tissue dry matter; a significant decrease in allantoic sodium concentration; a significant increase in amniotic fluid volume.

5.   Evidence is presented to suggest that the fetal system responds to sodium depletion in a manner similar to the maternal system.

6.   It is suggested that fetal urine contributes to both the amniotic and allantoic fluids.

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APPENDIX A

MATHEMATICAL ANALYSIS OF CLOSED, STEADY-STATE,

TWO COMPARTMENT SYSTEM

The analysis for the two compartment system was
that given by Sheppard (58). In these calculations the
rate of appearance of radioactivity in compartment 2 was
calculated rather than the rate of disappearance of
radioactivity from compartment 1. This was done to avoid
the difficulties, outlined in the text, of using compartment
1. Therefore solution to the equation:

$$P = \frac{dR_2/dt}{a_1 - a_2} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots(1)$$

would give the desired transfer rate,

where P = the transfer rate of sodium, in
mEq/hr.

dR_2/dt = The rate of change of total radioactivity
in compartment 2, in cpm/hr.

a_1 = Specific activity of compartment 1 at time
t_1, in cpm/mEq Na.

a_2 = Specific activity of compartment 2 at time
t_1, in cpm/mEq Na.

Transfer rates were calculated using four models of
a two compartment system. The first model assigned the compartments
as follows: compartment 1, the maternal exchangeable sodium pool;
compartment 2, the sodium pool of the total conceptus. The
parameter dR_2/dt was obtained by summing the rates of change
of total radioactivity in the fetus, amniotic and allantoic
fluid (Table XX and XXI). The parameter a_1 was the observed
value of the maternal sodium specific activity at time

t_1. The parameter a_2 was calculated from the expression (58).

$$a_2 = R/S$$

where a_2 = Specific activity in compartment 2 at time t_1, in cpm/mEq Na.

R = The total radioactivity of compartment 2, fetus, amniotic, and allantoic fluid, at time t_1, in cpm.

S = The total sodium pool of compartment 2, in mEq Na.

The results using this model are given in Table IV.

The second model used in the estimate of the sodium transfer rate was made between compartments designated compartment 1, the maternal exchangeable sodium pool; compartment 2, the fetus. Equation 1 was again used where the value for dR_2/dt was that value given for the fetus in Tables XX and XXI. The values of a_1 and a_2 were those values observed for the maternal and fetal plasma respectively at time t_1 (Table XX and XXI). The results using this model are given in Table V.

The third model used in estimating the sodium transfer rate using a two compartment system was accomplished assigning the compartments as follows: compartment 1, the maternal plus the fetal sodium pools; compartment 2, the amniotic plus the allantoic fluid sodium pools. This model was essentially that used by Friedman et al (24) in their studies on the transfer rate of sodium between the amniotic fluid and the maternal plus

fetal systems in the Rhesus monkey. Again equation 1 was used and the value for dR_2/dt was the sum of the rates of change of total radioactivity in the amniotic and allantoic fluid (Table XX and XXI). The value a_1 was that value of the maternal sodium specific activity at time t_1. The value a_2 was calculated using equation 2 where R was the total radioactivity in the amniotic plus allantoic fluid at time t_1 and S was the sum of the sodium pools of the amniotic and allantoic fluid. The results using this model are given in Table VI.

Model 4 as calculated was very similar to model 3. The difference was the value of a_1, the specific activity in compartment 1. The value a_1 was the value for the fetal plasma specific activity given in Tables XX and XXI. This differs from model 3 in that model 4 assumes that sodium enters the amniotic and allantoic fluids via the fetus. Model 3 assumes that sodium enters the amniotic and allantoic fluid directly from the maternal system. The results using model 4 are given in Table VII.

TABLE IV. Data of model 1 showing rate
of change of total radioactivity in compartment
2 (dR_2/dt), specific activity of compartments
1 and 2 at time t_1 (a_1 and a_2 respectively) and
sodium transfer rate (P).

Ewe No.	dR_2/dt (cpm/hr)	a_1 (cpm/mEq Na)	a_2 (cpm/mEq Na)	P (mEq/hr)
Control				
DC-2	158,460	23,735	9,592	11.20
DS-5	57,212	23,994	9,386	3.91
DS-11	166,747	22,193	7,893	11.66
DS-25	200,244	22,849	7,346	12.91
Mean				9.92
Standard error				2.03
Depleted				
DC-17	278,746	43,380	18,445	11.18
DS-27	54,422	8,982	2,924	8.98
DS-29	52,792	11,651	5,020	10.52
DS-37	117,517	35,297	16,275	6.17
Mean				9.21
Standard Error				1.11

TABLE V. Data of model 2 showing rate of change of total radioactivity in compartment 2 (dR_2/dt), specific activity of compartments 1 and 2 at time t_1 (a_1 and a_2 respectively) and sodium transfer rate (P).

Ewe No.	dR_2/dt (cpm/hr)	a_1 (cpm/mEq Na)	a_2 (cpm/mEq Na)	P (mEq/hr)
Control				
DC- 2	132,344	23,735	12,816	12.12
DS- 5	24,608	23,994	12,491	2.14
DS-11	91,370	22,193	10,630	7.90
DS-25	82,574	22,849	9,308	6.10
Mean				7.06
Standard Error				2.07
Depleted				
DC-17	196,027	43,380	21,735	9.06
DS-27	35,644	8,982	3,466	6.46
DS-29	5,440	11,651	6,354	1.03
DS-37	77,613	35,297	18,655	4.66
Mean				5.30
Standard Error				1.68

TABLE VI.   Data of model 3 showing rate of change of total radioactivity in compartment 2 (dR_2/dt), specific activity of compartments 1 and 2 at time t_1 (a_1 and a_2 respectively) and sodium transfer rate (P).

Ewe No.	dR_2/dt (cpm/hr)	a_1 (cpm/mEq Na)	a_2 (cpm/mEq Na)	P (mEq/hr)
Control				
DC- 2	26,116	23,735	1,656	1.18
DS- 5	32,604	23,994	2,542	1.52
DS-11	75,377	22,193	2,400	3.81
DS-25	117,670	22,849	3,619	6.12
Mean				3.16
Standard Error				1.16
Depleted				
DC-17	82,720	43,380	10,726	2.53
DS-27	18,778	8,982	2,094	2.72
DS-29	47,352	11,651	1,618	4.72
DS-37	39,904	35,297	12,164	1.72
Mean				2.92
Standard Error				0.64

TABLE VII. Data of model 4 showing rate of change of total radioactivity in compartment 2 (dR_2/dt), specific activity of compartments 1 and 2 at time t_1 (a_1 and a_2 respectively) and sodium transfer rate (P).

Ewe No.	dR_2/dt (cpm/hr)	a_1 (cpm/mEq Na)	a_2 (cpm/mEq Na)	P (mEq/hr)
Control				
DC- 2	26,116	12,816	1,656	2.34
DS- 5	32,604	12,491	2,542	3.28
DS-11	75,377	10,630	2,400	9.16
DS-25	117,670	9,308	3,619	20.68
Mean				8.86
Standard Error				4.21
Depleted				
DC-17	82,720	21,735	10,726	7.45
DS-27	18,778	3,466	2,094	13.69
DS-29	47,352	6,354	1,618	9.99
DS-37	39,904	18,655	12,164	6.15
Mean				9.32
Standard Error				1.67

APPENDIX B

TABLE VIII. Maternal Plasma sodium
and potassium concentrations in control
and depleted ewes. (mEq/l.)

Ewe No.	Sodium	Potassium
Control		
DC-2	150.0	4.57
DS-5	152.5	3.45
DS-11	150.0	3.86
DS-25	139.5	5.92
DS-31	161.0	4.68
DS-32	139.0	3.95
DS-45	151.0	4.76
Depleted		
DS-10	130.0	4.92
DC-17	130.7	5.13
DS-27	146.5	4.23
DS-29	134.7	4.31
DS-37	145.5	5.59

TABLE IX . Maternal data; body weight
(Kgm.) and exchangeable sodium pool size
(mEq and mEq/100 gm. B.W.) for control
and depleted ewes.

Ewe No.	Weight (Kgm.)	Exchangeable Na (mEq)	Exchangeable Na (mEq/100 gm.B.W.)
Control			
DC-2	56.75	2106.1	3.71
DS-5	63.56	2184.4	3.44
DS-11	71.73	2403.6	3.35
DS-25	71.73	2163.9	3.02
Depleted			
DS-10	60.38	1375.2	2.28
DC-17	—	1440.5	—
DS-27	58.11	1976.4	3.40
DS-29	66.74	2293.0	3.43
DS-37	57.20	1911.6	3.34

TABLE X.   Fetal plasma sodium and
potassium concentrations in control
and depleted groups (mEq/l).

Ewe No.	Sodium	Potassium
DC- 2	122.5	− 4.36
DS- 5	144.5	5.10
DS-11	147.5	5.84
DS-25	146.0	6.42
DS-31 I	142.7	5.22
DS-31 II	148.0	7.66
DS-32 I	151.5	5.66
DS-32 II	145.0	5.44
DS-45	152.5	5.84
Depleted		
DS-10	116.5	13.72
DC-17	147.0	4.94
DS-27	146.0	5.57
DS-29	142.7	6.78
DS-37	143.5	5.10

TABLE XI.   Minced fetal tissue sodium and potassium concentrations (mEq/100 gm) for control and depleted ewes.

Ewe No.		Sodium	Potassium
DC- 2		8.56	4.50
DS- 5		8.30	4.41
DS-11		8.50	4.26
DS-25		8.70	4.73
DS-31	I	8.85	4.61
DS-31	II	9.02	4.26
DS-32	I	8.69	4.13
DS-32	II	8.63	4.11
DS-45		8.24	4.28
Depleted			
DS-10		7.32	3.99
DC-17		8.42	4.80
DS-27		8.90	4.39
DS-29		8.35	4.40
DS-37		8.55	4.56

TABLE XII. Whole minced fetal tissue dry
matter ^{a}(%) for control and depleted ewes.

Control		Depleted	
Ewe No.	Dry Matter	Ewe No.	Dry Matter
DC- 2	21.59	DS-10	24.78
DS- 5	22.43	DC-17	24.06
DS-11	20.77	DS-27	23.83
DS-25	21.86	DS-29	22.55
DS-31 I	21.16	DS-37	23.22
DS-31 II	21.30		
DS-32 I	19.73		
DS-32 II	20.42		
DS-45	22.21		

a: dried in a lyophilizer for 149.5 hours at a pressure
less than 75 microns mercury.

TABLE XIII.   Fetal data; fetal body weight
(gms.) and exchangeable sodium pool size (mEq
and mEq/100 gm. FBW[*]) for control and depleted
ewes.

Ewe No.		Fetal Body Weight (gms.)	Exchangeable Na. (mEq)	Exchangeable Na (mEq/100 gm. FBW)
Control				
DC- 2		4590	392.9	8.56
DS- 5		3694	306.8	8.30
DS-11		3920	333.2	8.50
DS-25		4990	434.1	8.70
DS-31	I	4046	358.1	8.85
DS-31	II	2638	237.9	9.02
DS-45		3754	309.3	8.24
Depleted				
DS-10		4265	312.2	7.32
DC-17		4255	358.3	8.42
DS-27		3804	338.5	8.92
DS-29		4137	345.4	8.35
DS-37		3646	311.7	8.55

[*] Fetal Body Weight

TABLE XIV. Fetal adrenal weight expressed
in grams and as function of fetal body weight
for control and depleted groups.

Ewe No.		Fetal Adrenal Weight (gms.)	Fetal Adrenal Weight (gms./Kgm. FBW[a])
Control			
DC-2		0.4143	0.090
DS-5		.4486	.121
DS-11		.4762	.121
DS-25		.5344	.107
DS-31	I	.6497	.161
DS-31	II	.4101	.155
DS-45		.4468	.119
Depleted			
DS-10		.7545	.177
DC-17		.5979	.140
DS-27		.5687	.149
DS-29		.5650	.136
DS-37		.3178	.087

a: FBW = Fetal Body Weight

TABLE XV. Amniotic fluid sodium and
potassium concentrations (mEq/l.) for
control and depleted groups (including
twin data).

Ewe No.		Sodium	Potassium
Control			
DC- 2		97.0	9.06
DS- 5		94.2	6.47
DS-11		103.5	5.10
DS-25		130.5	3.34
DS-31	I	95.0	3.82
DS-31	II	99.5	7.48
DS-32	I	134.0	5.75
DS-32	II	118.0	4.67
DS-45		200.5	13.14
Depleted			
DS-10		65.5	5.67
DC-17		76.0	11.06
DS-27		127.7	3.93
DS-29		90.0	20.87
DS-37		128.0	4.17

TABLE XVI. Allantoic fluid sodium and potassium concentration (mEq/l) for control and depleted groups (including twin data).

Ewe No.		Sodium	Potassium
Control			
DC- 2		104.0	47.10
DS- 5		78.0	39.25
DS-11		83.0	4.25
DS-25		82.5	57.7
DS-31	I	46.0	50.0
DS-31	II	41.0	55.75
DS-32	I	75.5	46.10
DS-32	II	82.2	32.62
DS-45		71.5	5.72
Depleted			
DS-10		41.0	26.5
DC-17		29.0	87.0
DS-27		85.7	28.9
DS-29		24.0	84.2
DS-37		49.5	42.0

TABLE XVII. Amniotic and allantoic fluid
volumes (mls.) for control and depleted groups
(including twin data).

Ewe No.	Amniotic fluid	Allantoic fluid
Control		
DC- 2	901	695
DS- 5	1,163	380
DS-11	927	845
DS-25	1,129	985
DS-31 I	963	520
DS-31 II	933	525
DS-32 I	705	400
DS-32 II	668	382
Depleted		
DS-10	2,420	1455
DC-17	1,669	895
DS-27	1,908	565
DS-29	1,678	820
DS-37	1,231	462

DS-45 Omitted from control group due to mixing of fluids during
collection.

TABLE XVIII. Equations of regression lines of maternal plasma specific activity (cpm/mEq Na) with time (hrs.) for control and depleted groups (of form $Y = a + bx$)

Ewe No.

Control

DC- 2	$Y =$	23,839	$- 4.26x$
DS- 5	$Y =$	30,243	$- 257x$
DS-11	$Y =$	25,463	$- 133x$
DS-25	$Y =$	29,503	$- 274x^{**}$
DS-31	$Y =$	25,257	$- 194x$
DS-45	$Y =$	27,476	$- 47x$

Depleted

DS-10	$Y =$	54,771	$- 467x$
DC-17	$Y =$	45,551	$- 60x$
DS-27	$Y =$	10,131	$- 32x$
DS-29	$Y =$	16,235	$- 129x^{**}$
DS-37	$Y =$	40,250	$- 134x$

** Significant "b" ($P < .01$)

TABLE XIX. Whole minced fetal tissue
specific activity (cpm/mEq.Na) of control
and depleted groups.

Control		Depleted	
Ewe No.	Specific activity	Ewe No.	Specific activity
DC- 2	9,347	DS-10	17,336
DS- 5	11,773	DC-17	23,048
DS-11	10,380	DS-27	3,675
DS-25	8,133	DS-29	5,812
DS-31 I	8,537	DS-37	16,592
DS-31 II	6,774		
DS-45	10,954		

TABLE XX. Data of control group showing specific activity [1],
a (cpm/mEq Na.); rate of change of specific activity, da/dt
(cpm/mEq Na/hr); compartment size, S (mEq Na); rate of change
of total radioactivity, dR/dt (cpm/hr); and time of sampling after
injection for the four compartments outlined in the text.

Compartment No.	Time (hr.)	a (cpm/mEq.Na)	da/dt (cpm/mEq Na/hr.)	S (mEq Na)	dR/dt (cpm/hr.)
1	24.5	23,735			
	29.25	23,714	−4.42	2,106.1	−9,312
2	24.5	12,816			
	29.25	14,416	336.84	392.9	132,344
3	24.5	2,722			
	29.25	4,062	282.1	87.4	24,655
4	24.5	375			
	29.25	471	20.2	72.3	1,461
1	24.25	23,994			
	29.25	22,706	−257.6	2,184.4	−562,701
2	24.5	12,491			
	29.25	12,872	80.2	306.8	24,608
3	24.75	2,928			
	29.25	4,095	259.3	109.6	28,425
4	24.25	1,115			
	29.25	1,820	141.0	29.6	4,179

[1] Specific activity of compartment 1 was interpolated from the equation
of regression line of the maternal plasma specific activity.

TABLE XX   – continued

Compartment No.	Time (hr.)	a (cpm/mEq Na)	da/dt (cpm/mEq Na/hr.)	S (mEq Na)	dR/dt (cpm/hr.)
11 1	24.5	22,193			
	29.25	21,560	−133.3	2,403.6	−320,304
2	24.75	10,630			
	29.25	11,864	274.2	333.2	91,370
3	25.0	3,449			
	29.25	4,618	275.1	95.9	26,389
4	24.5	964			
	29.25	4,282	698.5	70.1	48,988
25 1	24.25	22,849			
	28.75	21,614	−274.4	2,163.9	−593,861
2	24.25	9,308			
	28.75	10,164	190.2	434.1	82,574
3	24.25	5,222			
	28.75	7,686	547.5	147.3	80,670
4	24.25	715			
	28.75	2,764	455.3	81.3	37,000

TABLE XX - continued

Compartment No.		Time (hr.)	a (cpm/mEq Na)	da/dt (cpm/mEq Na/hr.)	S (mEq Na)	dR/dt (cpm/hr.)
31[2]	1	25.0	20,408			
		29.75	19,486	-194.1	--	--
	2 I	25.25	10,228			
		29.75	10,326	21.7	358.1	7,771
	3 I	25.0	3,000			
		29.75	5,874	605.0	91.5	55,357
	4 I	25.0	1,652			
		29.75	1,956	64.0	23.9	1,529
45[3]	1	25.0	26,302			
		29.75	26,079	-46.9	--	--
	2	25.0	11,692			
		29.75	12,872	248.4	309.3	76,830
	3	25.0	3,551			
		29.75	4,464	202.9	--	--
	4	25.0	10,181			
		29.75	14,601	982.2	--	--

[2] Data of compartments 2,3 and 4 is for fetus I; fetus II was not sampled at time t_1. Therefore no estimate of maternal exchangeable sodium could be made.

[3] Missing data is due to fetal fluid volumes not being obtained.

TABLE XXI. Data of sodium depleted ewes showing specific activity [1], a (cpm/mEq Na); rate of change of specific activity, da/dt (cpm/mEq Na/hr); compartment size, S (mEq Na); rate of change of total radio-activity, dR/dt (cpm/hr); and time of sampling after injection for the four compartments outlined in the text.

	Compartment No.	Time (hrs.)	a (cpm/mEq Na)	da/dt (cpm/mEq Na/hr.)	S (mEq Na)	dR/dt (cpm/hr)
-10	1	37.0	37,503			
		41.25	35,520	-466.6	1,375.2	-641,668
	2	37.0	19,322			
		41.25	22,069	646.3	312.2	201,775
	3	37.0	6,488			
		41.25	6,702	50.3	158.5	7,972
	4	37.0	9,638			
		41.25	17,681	1,892.5	68.4	129,447
-17	1	36.25	43,380			
		40.75	43,110	-60.0	1,440.5	-86,430
	2	36.25	21,735			
		40.75	24,197	547.1	358.3	196,026
	3	36.25	12,487			
		40.75	14,974	552.6	126.8	70,070
	4	36.25	2,103			
		40.75	4,301	488.4	25.9	12,650

[1] Specific activity of compartment 1 was interpolated from the equation of the regression line of the maternal plasma specific activity.

TABLE XXI — continued

Compartment No.	Time (hrs.)	$\bar{a}$ (cpm/mEq Na)	da/dt (cpm/mEq Na/hr)	$\bar{S}$ (mEq Na)	dR/dt (cpm/hr)
1	36.0	8,982			
	41.25	8,814	−32.0	1,976.4	−63,245
2	36.5	3,466			
	41.25	3,966	105.3	338.5	35,644
3	36.0	2,461			
	41.25	2,830	70.3	238.0	16,731
4	36.0	291			
	41.25	513	42.3	48.4	2,047
1	35.5	11,651			
	39.5	11,135	−129.9	2,293.0	−295,797
2	35.5	6,354			
	39.5	6,417	15.7	345.4	5,440
3	35.5	1,655			
	39.5	2,855	300.0	151.0	45,300
4	35.5	1,333			
	39.5	1,750	104.2	19.7	2,052
1	37.0	35,297			
	41.5	34,694	−134.0	1,911.6	−256,154
2	37.5	18,655			
	41.5	19,651	249.0	311.7	77,613
3	37.0	13,492			
	41.5	14,429	208.2	157.6	32,812
4	37.0	3,030			
	41.5	4,424	309.7	22.9	7,092